**BACTERIAL ENDOTOXIN TEST METHOD SUITABILITY PROTOCOL FOR PACLITAXEL**

**TABLE OF CONTENTS**

[1.0 OBJECTIVE: 3](#_Toc491091102)

[2.0 EXECUTION TEAM: 3](#_Toc491091103)

[3.0 PERSONNEL RESPONSIBILITIES: 3](#_Toc491091104)

[4.0 QUALIFICATION PRE REQUISITES: 4](#_Toc491091105)

5.0 qualification procedure:…………………………………………………………….4

[6.0 InCIDENTS / DISCREPANCIES RECORD:](#_Toc491091107) 8

[7.0 REFERENCE DOCUMENTS:](#_Toc491091108) 9

[8.0 ABBREVIATIONS:](#_Toc491091109) 9

[9.0 ANNEXURES:](#_Toc491091110) 10

[10.0 ATTACHMENTS:](#_Toc491091111) 10

[11.0 SUMMARY: 1](#_Toc491091112)1

1. **OBJECTIVE**

The objective of this protocol is to verify the Bacterial Endotoxin Test for Paclitaxel by gel-clot method to find out the Maximum valid dilution (MVD) and to perform the Inhibition and Enhancement test by using harmonized method at oncology block at Jodas Expoim Private Limited, plot No: 55, phase-3, Biotech Park, Karkapatla village.

Validation of three batches shall be performed at manufacturing site.

**Vendor Name:**

1. **EXECUTION TEAM:**
2. **Training to the executors:**

The executors shall be trained before the execution of protocol as per respective procedures and attach the training record.

1. **List of Executors Involved in Qualification Study:**

Record the details of the executors involved in the Qualification activity like name, department, designation and training details with signature and date in the Annexure-1.

1. **PERSONNEL RESPONSIBILITIES:**

Responsibilities of individual department / personnel while execution of protocol is as under:

**Microbiology**

* Responsible for preparation and review of protocol.
* Provision of training to all concerned persons on protocol prior to execution
* Execution of the protocol.
* Assist in the investigation of variances if any.
* Prepare the summary and conclusion of the activity.
* Head / Designee shall be responsible for approval, review of the protocol,

Summary & conclusion and certification of the protocol.

**QA-Validation**

* Responsible for review of protocol.
* Assist in the investigation of variances if any.
* Verifies that the test requirements described in protocol are meeting criteria for the performed tests and properly documented.

**Quality Assurance**

* Quality Assurance Head / Designee shall be responsible for approval, review

Of the protocol, summary & conclusion and certification of the protocol.

1. **QUALIFICATION PRE REQUISITES:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No.** | **Description** | **Yes/No/NA** | **Checked by(QM)** | **Verified**  **by (QM)** |
|  | Training is imparted to personnel responsible to execute the study. |  |  |  |
|  | Others if any \_\_\_\_\_\_\_\_\_\_\_\_\_. |  |  |  |

**Note:** The above pre-requisites should be verified and if complete general study protocol activity shall be carried out.

**Comments:**

**Reviewed by (QA): Date:**

1. **QUALIFICATION PROCEDURE:**
   1. **Requirements:**

* LyophilizedLAL reagent
* Control Standard Endotoxin (CSE)
* LAL Reagent Water (LRW)
* Samples for Bacterial Endotoxin Test
  1. **Materials and equipments:**
* Vortex Mixer
* 10 x 75 mm depyrogenated test tubes.
* 13 x 100 mm depyrogenated glass tubes for dilution of CSE.
* Calibrated heating block set at 370C ± 10C.
* Calibrated Micropipette.
* Depyrogenated disposable tips (use fresh tips only do not recycle).
* Stop Watch
* Test Tube rack

**Limits:**

|  |  |  |
| --- | --- | --- |
| **S.No** | **Name of the Products** | **BET Limit** |
| 1 | Paclitaxel | NMT 0.4 EU/mg of Paclitaxel |

* 1. **Determination of Maximum Valid Dilution:**

MVD shall be determined based on the formula and shall be calculated as per Annexure 2.

* 1. **Preparation of Solutions:**

Carry out the preparation of CSE and Sample Solution as follows.

1. **Preparation of Control Standard Endotoxin Solutions (CSE):**
2. Reconstitute the lyophilized Control Standard Endotoxin (E.coli) with required quantity of LRW (Mentioned in manufacturer COA) with the help of pyrogen free pipette tips. After rehydration, vortex vigorously follows the manufacturer’ instructions as per COA of CSE.
3. If required dilute it further with LRW to get different known concentrations. Mix vigorously the reconstituted CSE vial, using a vortex mixture as per vendor recommendation and follow the manufacturer’s instructions as per COA, before making appropriate serial dilutions.
4. Carry out the dilutions as per attachment -1, from obtained concentration:1000 EU/mL
5. Vortex each tube before making serial dilutions as per vendor recommendation.
6. **Preparation of LAL Reagent:**
7. Reconstitute the lyophilized powder using required quantity of LRW as per manufacturer COA with the help of depyrogenated micropipette tips.

Note: Do not vortex, invert or shake vigorously.

1. **Confirmation of labelled lysate sensitivity:**
2. Confirm in four replicates the labeled sensitivity, λ, expressed in EU/mL of the lysate prior to use in the method suitability.
3. Prepare standard solutions having at least four concentrations equivalent to 2λ, λ, λ/2, and λ/4 by diluting with LAL reagent water.
4. Mix a volume of the Lysate with an equal volume (such as 0.1-mL aliquots) of one of the Standard Endotoxin Solutions in each test tube.
5. Incubate the reaction mixture for a constant period according to the directions of the lysate manufacturer (usually at 37 ± 1° for 60 ± 2 min), avoiding vibration.
6. To test the integrity of the gel, take each tube in turn directly from the incubator, and invert it through about 180° in one smooth motion.
7. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if an intact gel is not formed. The test is considered valid when the lowest concentration of the standard solutions shows a negative result in all replicate tests.
8. The endpoint is the smallest concentration in the series of decreasing concentrations of standard endotoxin that clots the lysate. Determine the geometric mean endpoint by calculating the mean of the logarithms of the endpoint concentrations of the four replicate series and then taking the antilogarithm of the mean value, as indicated in the following formula:

Geometric mean endpoint concentration = antilog (Σ*e*/*f*)

1. Where Σ*e* is the sum of the log endpoint concentrations of the dilution series used, and *f* is the number of replicate test tubes. The geometric mean endpoint concentration is the measured sensitivity of the lysate (in EU/mL). If this is not less than 0.5λ and not more than 2λ, the labeled sensitivity is confirmed and is used

in tests performed with this lysate.

1. Record the results in Annexure -3.
2. **Preparation of Sample solution:**

Product Name : Paclitaxel

Endotoxin limit : 0.4 EU/mg

Amount of sample taken 100 mg. Consider the tube as “A”.

Volume of LRW added to tube “A”: 1mL of LRW

Vortex the tube for 2 minutes. Final concentration of sample in tube “A” is 100mg/mL

Follow the dilution series (Each tube vortex at least 2 minutes before dilution) as mentioned in Attachment – 2.

Record the sample preparation details in Annexure – 4.

1. **Screening for Interference:**
2. Label and arrange test tubes (10 x 75 mm) in test tube stand and label them as per the tube numbers mentioned in annexure-5 for pH verification.
3. pH of the sample solution to be examined (mixture of the lysate and sample solution) shall be within the range of 6.0 to 8.0 (Adjust the pH of the sample if required).
4. Record the results of pH in Annexure-5.
5. Label and arrange the tubes for negative control and sample dilutions for determination of Non inhibitory dilution (NID) as per attachment-3 and perform the test in duplicates.
6. **Incubation and Observation:**
7. Incubate the reaction mixture tubes at 37 ± 1°C for 60 ± 2 minutes or according to the directions of the Lysate manufacturer, avoiding vibration.
8. After Incubation take each tube directly from the heat block, and invert it through about 180° in one smooth motion.
9. If a firm gel has formed that remains in place upon inversion, record the result as positive.
10. A result is negative if an intact gel is not formed. Record the results of determination of non-inhibitory dilution in Annexure - 5.
11. **Selection of dilution for Inhibition and Enhancement test:**
12. Perform the inhibition and Enhancement test by selecting the dilution where PPC is showing positive result and NPC is showing negative result (NID) not exceeding the MVD.
13. **Inhibition & Enhancement Test:**
    * 1. Label and arrange test tubes (10x75mm) in test tube stand and add LRW, sample, prepared Control Standard Endotoxin and Lysate quadruplicate as per the table given in attachment – 4.
      2. On the day of Inhibition and enhancement test document the CSE dilution details and sample preparation details in annexure-4.

**Solution A:** Sample solution at selected MVD.

**Solution B:** Sample solution spiked with indicated CSE concentrations.

(Positive product Control: PPC).

**Solution C:** Standard solution which indicated CSE concentration in LRW

**Solution D:** Negative Control of LRW (NC).

After Incubation and observation of the all replicate tubes, determine the geometric mean endpoint by calculating the mean of the logarithms of the endpoint concentrations of the four replicate series and then taking the antilogarithm of the mean value, as indicated in the following formula and record the results in Annexure-6.

Geometric Mean Endpoint Concentration = antilog (Σe/f)

Where Ʃe is the sum of the log endpoint concentrations of the solution B and C, and f is the number of replicate test tubes.

1. **Acceptance Criteria:**
   * 1. The Inhibition and Enhancement test is considered valid when all the replicates of Solution A and D show Negative Result.
     2. The result of solution C confirm the labeled sensitivity of the Lysate it should not be greater than 2λ and not less than λ/2.
     3. The geometric mean of the end point concentration of solution B should not be greater than 2λ and not less than λ/2.
     4. Record the observations of Inhibition and enhancement test in annexure-6.
2. **INCIDENTS / DISCREPANCIES RECORD:**

List the Incidents/discrepancies observed if any during the execution of protocol and evaluate the Incident / discrepancy as per the SOP and implement the corrective actions for the same and attach the Incident / discrepancy record to this protocol.

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| **Sr. No.** | **Description of the Incident / Discrepancy** |
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1. **REFERENCE DOCUMENTS:**

IP 2.2.3

1. **ABBREVIATIONS:**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Abbreviations** | **Description** |
|  | SOP | Standard Operating Procedure |
|  | QA | Quality Assurance |
|  | QM | Quality control |
|  | LAL | Limulus Amoebocyte Lysate |
|  | COA | Certificate of Analysis |
|  | EU | Endotoxin Unit |
|  | mg | milligram |
|  | mL | milliliter |
|  | LRW | LAL Reagent water |
|  | CSE | Control Standard Endotoxin |
|  | NID | Non Interfering Dilution |
|  | PPC | Positive Product Control |
|  | NMT | Not More Than |
|  | MVD | Maximum Valid Dilution |
|  | NPC | Negative Product Control |
|  | Ph.Eur | European Pharmacopeia |
|  | NID | Non Inhibitory dilution |

1. **ANNEXURES:**

|  |  |  |
| --- | --- | --- |
| Annexure-1 | : | Details of the executors |
| Annexure-2 | : | Determination of maximum valid dilution test record |
| Annexure-3 | : | Confirmation of labeled lysate sensitivity |
| Annexure-4 | : | Preparation of solutions record |
| Annexure-5 | : | Screening for interference test record |

Annexure-6 : Inhibition and Enhancement test record

1. **ATTACHMENTS:**

| **Sr. No.** | **Title** |
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1. **SUMMARY:**

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| --- | --- | --- | --- |
| **Description** | **Yes/No/NA** | **Checked**  **by (QM)** | **Verified**  **by (QM)** |
| All acceptance criteria set forth in the protocol were met. |  |  |  |
| All specifications, data sheets were signed as checked and verified. |  |  |  |
| Incidents if any |  |  |  |
| Whether acceptable |  |  |  |
| If not acceptable, action taken |  |  |  |

**END OF THE DOCUMENT**